

## Composition and Larvicidal Action of *Ocimum basilicum* L. Essential Oil against *Spodoptera littoralis* (Boisd.)

Hala M. I. Mead

Plant Protection Research Institute, Agricultural Research Center, Doqqi, Giza, Egypt.



### ABSTRACT

This study aimed to evaluate the bioactivity of the essential oil of basil leaves, *Ocimum basilicum* on newly hatched larvae of *Spodoptera littoralis*. The chemical composition of the basil oil using (GC-MS) analysis showed Linalool (48.26%), Eucalyptol (9.21%) and Estragole (5.16%) as the major constituents. Results indicated that the basil oil has larvicidal activity against *S. littoralis*. Latent effects with LC<sub>50</sub> value of basil oil on the successive stages were also detected, both larval and pupal duration was significantly elongated as compared to control, while the reverse was true in case of pupation and pupal weight. Additionally, the tested essential oil caused significant inhibition of the activity of acetylcholine esterase enzyme and significant increase in Lactate dehydrogenase enzyme than control.

**Keywords:** *Ocimum basilicum*; *Spodoptera littoralis*; larvicidal; Enzymes.

### INTRODUCTION

Pest control constitutes a major and ancient preoccupation of human beings. Phytophagous insects including cotton leafworm, *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae) damage cotton crops, causing the loss of more than 47% (Riba and Silvy 1989). In Egypt, *S. littoralis* reduced both quantity and quality of the attacked crops (Russel *et al.* 1993).

Recently, many researches have directed focus towards new and more effective bio insecticides which are environmentally safe (biodegradable) and based on plant extracts and oils Khedr (2016). Among the multitude of plant species, some of them are called aromatic plants, because it is commonly known the presence of essential oils which give them a characteristic flavor and a distinguished odour.

In the Mediterranean basin, many species of aromatic plants belonging to family Lamiaceae were grown wild (Martins *et al.* 1999). Most of them were used as traditional medicine, food spice and in perfumery industrial. Additionally, using the essential oils in insect pest management (IPM) recorded by Mead (2012) and Khedr & El-Kawas (2013). The essential oil of basil, *Ocimum basilicum* L. (Lamiaceae) is well extracted from the leaves by steam distillation. Such oil has ovicidal, larvicidal, physicochemical and repellent activities against major pests (Azhari *et al.* 2012 and Foko *et al.* 2016). Moreover, this oil has also antioxidant, antimicrobial and cytotoxic activity (Shirazi *et al.* 2014).

This alternative strategy could improve the biodegradability of insecticide treatments and subsequently decrease the quantity of insecticide residues, increase insecticide selectivity and develop a better respect for the environment. Thus, the objective of this study was to determine the chemical composition of the basil essential oil and its larvicidal action against *S. littoralis* larvae.

### MATERIALS AND METHODS

#### 1. Plant materials and isolation of its essential oil:

The essential oil was extracted from the leaves of basil, *Ocimum basilicum* L. of the family Lamiaceae. About 250 gm of basil leaves were bought from local market, Sharquia Governorate, Egypt, (30°34'00"N and 31°30'00"E). The essential oil was extracted using

a Clevenger-tube apparatus (Marcus and Lichtenstein 1979), where the basil leaves were subjected to hydro distillation for 24 hours. The basil oil was separated, dried over anhydrous sodium sulfate to remove water after extraction and stored in dark glass bottles at 4 °C in a refrigerator until used. The isolated oil is a pale yellow liquid with a distinguished odor and taste of basil.

#### 2. GC-MS analysis of the essential oil:

The essential oil was analyzed on Gas Chromatography Mass Spectrometry (GC-MS) HP 6890 Series A (Agilent) at National Research Center, Giza, Egypt. The constituents of oil were identified using computer matching and comparing the fragmentation patterns of their masses with those listed by Adams (1989).

#### 3. Rearing techniques of *Spodoptera littoralis* larvae:

A laboratory strain of cotton leafworm, *S. littoralis* were reared in Plant Protection Research Institute, Zagazig, Egypt, under constant conditions of 27±1 °C and 65±5 % R.H. % according to El-Defrawi *et al.* (1964).

#### 4. Bioassay tests:

To study the larvicidal action of basil essential oil against newly hatched larvae (1<sup>st</sup> instar larvae) of *S. littoralis*, five serial concentrations of the essential oil were prepared using ethyl alcohol (95%) as a solvent (0.625, 1.25, 2.5, 5 and 10 %) (v/v). Leaf discs (3 cm. diameter) of the fresh castor bean leaves were bunched with a cork borer, then dipped in the tested concentrations for 10 seconds then dried, (leaf dip technique). Newly hatched larvae were transferred to the treated leaves. Control disks were dipped in ethyl alcohol (95%) only. Each tested concentration and control was represented by five replicates (20 newly hatched larvae/replicate). The larvae were allowed to feed on treated disks for 48 hours then on untreated ones. Mortality was recorded after 72 hours of treatment. Mortality percentages were corrected according to Abbott (1925) formula to estimate the LC values.

#### 5. Latent effects of basil essential oil on the successive stages of *S. littoralis* resulted from treated larvae:

The LC<sub>50</sub> value of basil essential oil against 1<sup>st</sup> instar larvae of *S. littoralis* was used to evaluate some biological parameters occurred in the successive stages resulted from treated larvae.

Three replicates were used for essential oil and control (20 newly hatched larvae/replicate). Selected larvae were starved for 4-6 hours, then transferred into treated and non-treated disks. The disks were changed after 48 hours with fresh leaves. Larvae were checked daily until pupation under laboratory conditions. Larval and pupal duration, pupation percentages and pupal weight have represented the parameters of long-term bioactivity of basil essential oil.

## 6. Biochemical assay:

### 1. Preparation of samples:

The preparation of samples involved the use of newly hatched larvae of *S. littoralis* after 72 hours of treatment with LC<sub>50</sub> of basil oil and control. The healthy larvae were picked up and placed in clean jars, then starved for 4 hr. Five milligrams of treatment and control were homogenized in distilled water using a chilled glass Teflon tissue homogenizer (ST-2 Mechanic-Preczyina, Poland) surrounded with a jacket of crushed ice for three minutes. The homogenates were centrifuged at 8000 r.p.m for 15 minutes at 5°C in a refrigerated microcentrifuge to remove haemocytes.

The supernatants were transferred to clean tubes and stored in freezer at -20°C until used. Three replicated were used for each biochemical assay for measuring the absorbance of colored substances or metabolic compounds, double beam ultraviolet/ visible spectrophotometer (Spectronic 1201, Milton Roy Co., USA).

### 2. Total protein assessment:

Total protein concentration was estimated according to Bradford (1976) using bovine serum albumin as a standard. Protein reagent was set by dissolving 100 mg of coomassie Brilliant blue G-250 (SIGMA) in 50 ml 95% ethanol. 100 ml of phosphoric acid 85% (w/v) were added to their solution. The resulting solution was diluted to obtain a final volume of 1 liter.

### 3. Determination of enzyme activities

#### Acetylcholine esterase (EC 3.1.1.7):

AChE activity was measured according to the method described by Simpson *et al.* (1964), which using acetylcholine bromide (AchBr) as a substrate. The reaction mixture contained 200 µl enzyme solution, 0.5 ml of 0.1µ phosphate buffer (pH 7.0) and 0.5 ml of 3 mM (AchBr).

#### Lactate dehydrogenase (LDH) (EC 1.1.1.27):

Lactate dehydrogenase (LDH) catalyzes the conversion of pyruvate to lactate. The rate of decrease in NADH is directly proportional to LDH activity that is determined photometrically at 340 nm. according to Pesce 1984 method. LDH catalyzed the reduction of pyruvate by NADH. The rate of decrease in concentration of NADH, measured photometrically proportional to the catalytic concentration of LDH present in the sample.

### 7. Statistical analysis:

Using the computed percentage of mortalities versus corresponding concentrations, Probit analysis was adopted according to Finney (1971). This yields the toxicity indices (LC<sub>50</sub> and LC<sub>90</sub>) as well as the related parameters (slope, and chi-square,  $\chi^2$ ) for established toxicity regression lines.

The data are presented as mean + SE and the statistical significance was analysed using Student's 't' test, P<0.05 was considered significant (Snedecor and Cochran 1980).

## RESULTS

### 1. Chemical constituents of basil, *O. basilicum* essential oil:

The chemical composition of basil essential oil is tabulated in Table 1 in the order of the retention times of the constituents. The GC-MS revealed 30 peaks corresponding to the main active ingredients of the oil (Figure 1). The three major active components were identified as: Linalool (48.26%), Eucalyptol (9.21%) and Estragole (5.16%), while Linalool oxide recorded the least one (0.37%).

### 2. Larvicidal tests:

Data in Table 2 showed that the essential oil of basil has a larvicidal effect on newly hatched larvae (1<sup>st</sup> instar larvae) of *S. littoralis* after 72 hours of treatment, where LC<sub>50</sub> and LC<sub>90</sub> were (1.176 and 9.08%), respectively.

### 3. Latent effects of basil essential oil on the successive stages of *S. littoralis* resulted from treated larvae:

#### 1. Larval duration:

Data in Table (3) indicated that using LC<sub>50</sub> of basil essential oil on *S. littoralis* larvae significantly elongated the larval duration from 17.12±0.16 days for control to 18.11±0.25 days, P=0.0301.

#### 2. Pupation percentage:

The result in Table (3) indicated that the treated larvae exhibited pupae by (90.00±1.155%) which recorded highly significant reduction than 100% pupae resulting from control, P=0.0010.

#### 3. Pupal duration:

Result in the same Table, showed that the pupal duration lasted 11.10±0.11 days for pupae developed from untreated larvae and 12.70±0.10 days for pupae developed from treated one. This increase was statistically high significant when compared to pupal control, P= 0.0006.

#### 4. Pupal weight:

The obtained results in Table 3 indicated that the basil essential oil reduced significantly the average weight of pupae developed from treated larvae (0.302±0.006 gm) than pupal control (0.3526±0.011 gm), P=0.015.

### 4. Biochemical assay:

#### 1. Acetylcholine esterase (AChE):

AChE activity showed significant inhibition after treatment with LC<sub>50</sub> value of basil essential oil (1.00±0.05 µg AchBr/ min/ mg protein), as compared to control (1.35±0.081 µg AchBr/ minutes/ mg protein), P=0.021, (Table 4).

#### 2. Lactate dehydrogenase (LDH):

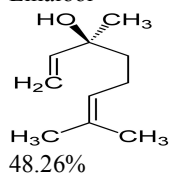
The basil essential oil caused significant elevation in the activity of LDH enzyme (15.15±0.95 U X 10<sup>3</sup>/mg protein) than control (11.48±0.72 U X 10<sup>3</sup>/mg protein), P= 0.043, (Table 4).

**Table 1. Chemical composition of essential oil of *O. basilicum* leaves.**

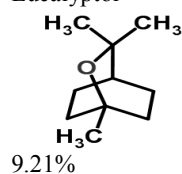
No.	Chemical Compounds	Molecular Formula	Molecular Weight	Retention Time	Area %
1	β-Myrcene	C <sub>10</sub> H <sub>16</sub>	136	9.39	2.98
2	α-Phellandrene	C <sub>10</sub> H <sub>16</sub>	136	10.08	0.51
3	α-Terpinene	C <sub>10</sub> H <sub>16</sub>	136	10.45	1.08
4	Limonene	C <sub>10</sub> H <sub>16</sub>	136	10.91	2.00
5	Eucalyptol	C <sub>10</sub> H <sub>18</sub> O	154	11.06	9.21
6	trans-β-Ocimene	C <sub>10</sub> H <sub>16</sub>	136	11.54	1.41
7	γ-Terpinene	C <sub>10</sub> H <sub>16</sub>	136	12.03	0.90
8	Linalool oxide	C <sub>10</sub> H <sub>18</sub> O <sub>2</sub>	170	12.52	0.37
9	p-Mentha-1,4(8)-diene	C <sub>10</sub> H <sub>16</sub>	136	13.06	1.10
10	p-Menth-1-en-8-ol	C <sub>10</sub> H <sub>18</sub> O	154	13.15	0.54
11	Linalool	C <sub>10</sub> H <sub>18</sub> O	154	13.66	48.26
12	Camphor	C <sub>10</sub> H <sub>18</sub> O	152	15.84	0.92
13	Borneol	C <sub>10</sub> H <sub>18</sub> O	154	16.81	1.04
14	Terpinene-4-ol	C <sub>10</sub> H <sub>18</sub> O	154	17.09	2.53
15	Linalyl propionate	C <sub>13</sub> H <sub>22</sub> O <sub>2</sub>	210	17.78	0.78
16	Estragole	C <sub>10</sub> H <sub>12</sub> O	148	18.00	5.16
17	Bornyl acetate	C <sub>12</sub> H <sub>20</sub> O <sub>2</sub>	196	21.04	1.59
18	β-Elemene	C <sub>15</sub> H <sub>24</sub>	204	24.96	0.61
19	Aromandendrene	C <sub>15</sub> H <sub>24</sub>	204	25.55	0.52
20	trans-Caryophyllene	C <sub>15</sub> H <sub>24</sub>	204	26.14	0.57
21	α-Bergamotene	C <sub>15</sub> H <sub>24</sub>	204	26.52	3.47
22	β-ylangene	C <sub>15</sub> H <sub>24</sub>	204	27.53	1.00
23	Valencene	C <sub>15</sub> H <sub>24</sub>	204	28.81	1.15
24	β-copaene	C <sub>15</sub> H <sub>24</sub>	204	29.02	0.71
25	Junipene	C <sub>15</sub> H <sub>24</sub>	204	29.16	0.60
26	γ-Cadinene	C <sub>15</sub> H <sub>24</sub>	204	29.64	3.91
27	γ-Murolene	C <sub>15</sub> H <sub>24</sub>	204	30.50	0.80
28	Spathulenol	C <sub>15</sub> H <sub>24</sub> O	220	32.13	0.31
29	Cubenol	C <sub>15</sub> H <sub>26</sub> O	222	33.38	0.82
30	Cadinol	C <sub>15</sub> H <sub>26</sub> O	222	34.35	5.15

Major components structure

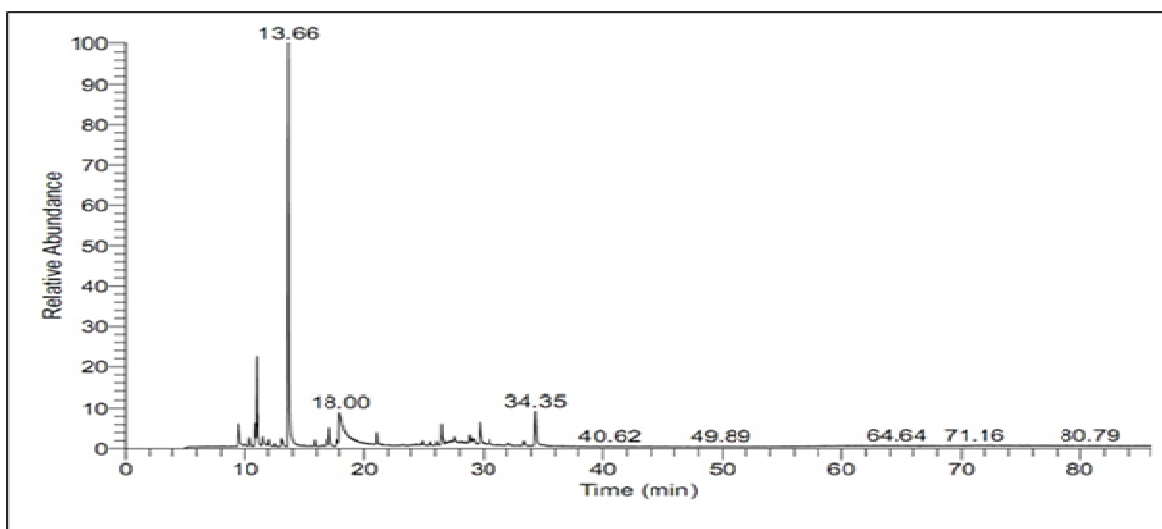
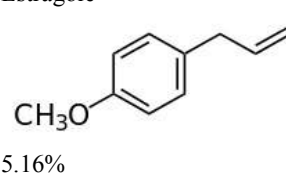
Linalool



Eucalyptol



Estragole



**Fig. 1. Gas chromatography profile of the essential oil of *O. basilicum* leaves.**

**Table 2. Larvicidal action of basil, *O. basilicum* essential oil on 1<sup>st</sup> instar larvae of *S. littoralis* after 72 hr of treatment.**

Treatment	LC <sub>50</sub> (%) (Lower-Upper)	LC <sub>90</sub> (%) (Lower-Upper)	Slope ± SE	X <sup>2</sup>
Basil essential oil	1.176 (0.36 – 1.827)	9.08 (7.846-37.32)	1.444±0.155	10.22

**Table 3. Biological aspects of *S. littoralis* treated with the essential oil of basil, *O. basilicum*.**

Treatments	Larval duration (days)	Pupation %	Pupal weight (gm)	Pupal duration (days)
Control	17.12±0.16	100±0.00	0.3526±0.011	11.10±0.11
Basil oil	18.11±0.25	90.00±1.15	0.3020±0.006	12.70±0.10
P	0.0301	0.0010	0.0151	0.0006

Notes: Each datum represents the mean of three replicates.

-Data expressed as Mean ± Standard Error (SE). Significance different (P <0.05), highly significant (P <0.01).

-Treated larvae at level of LC<sub>50</sub> of basil essential oil.

**Table 4. Changes in some biochemical parameters in *S. littoralis* larvae treated with basil, *O. basilicum* essential oil.**

Treatments	Acetyl choline esterase (AChE) µg AchBr/ min/ mg protein	Lactate dehydrogenase (LDH) U X 10 <sup>3</sup> mg protein
Control	1.35±0.08	11.48±0.72
Basil oil	1.00± 0.05	15.15±0.95
P	0.021	0.043

Notes: Each datum represents the mean of three replicates.

-Data expressed as Mean ± Standard Error (SE). Significance different (P <0.05), highly significant (P <0.01).

-Treated larvae at level of LC<sub>50</sub> of basil essential oil.

## DISCUSSION

In this experiment, GC-MS analysis of basil leaves, *Ocimum basilicum* essential oil revealed 30 peaks corresponding to the main active ingredients of this oil. In recent studies using the same tested plant, Shirazi *et al.* (2014) and Foko *et al.* (2016) obtained 25 and 29 chemical compounds, respectively. Whereas, Azhari *et al.* (2012) obtained 13 compounds although these authors used the same plant. The variations in the chemical constituents of basil essential oil may be attributed mainly to the physiological development of plant and its degree of maturity, seasonal variations, climate and soil conditions, part of plant used and the method of harvesting or the method used to isolate the plant product (Misharina 2001 and Smallfield *et al.* 2001). Moreover, Lawrence *et al.* (1988) in a study of basil essential oil of different geographical origins, found that the main constituents are produced by two different biochemical pathways, the terpenes (linalool and geraniol) using mevalonic acid pathway and phenyl propanoids (methyl chavicol, eugenol and methyleugenol) by Shikimic acid pathway.

Monoterpenes were found as the main components of *O. basilicum* essential oil. The same conclusion was obtained by Dolatabad *et al.* (2014) when tested four *Ocimum* species growing in Iran. Also Foko *et al.* (2016) found that monoterpenes were the main components of *O. basilicum* that obtained (84.3%). The active components were Linalool (48.26%) Eucalyptol (9.21%) and Estragole (5.16%). In Egypt, Abou El-Soud *et al.* (2015) demonstrated Linalool is an active constituent compound in Basil essential oil. Reversely, Foko *et al.* 2016 reported that, basil oil was characterized by its high content of 1-8 cineol (33.90%), β-pinene (16.09%) as the main ingredients while Linalool gave (0.62%). Whereas, Shirazi *et al.* (2014) found that methylchavicol (46.90%) and geraniol (19.10%) were the major chemical components.

Based on LC<sub>50</sub> and LC<sub>90</sub> values, the basil essential oil proved to possess larvicidal action against newly hatched

larvae (1st instar larvae) of *S. littoralis* using leaf dip technique. Basil oil exerted larvicidal activity against different insects (Azhari *et al.* 2012 and Foko *et al.* 2016). Additionally, López *et al.* (2008) and Badawy *et al.* (2010) found that Linalool is responsible for the toxicity against different pests. (Bakkali *et al.* 2008) stated that, Monoterpenes can penetrate cell walls, cytoplasmic membrane and interfere with the physiological processes of insects and subsequently lead to mortality.

The mechanism of toxic effect on the essential oil of basil leaves on *S. littoralis* larvae may be due to the neurotoxicity of several monoterpenes (Linalool, myrcene, limonene and terpineol) which have been identified as important components of basil oil (Coats *et al.* 1991). In this study, basil oil caused significant inhibition of Acetylcholin esterase (AChE) and significant elevation in Lactate dehydrogenase (LDH) enzyme activity as compared to control. Many reports indicate that monoterpenoids of the essential oil especially linalool causing inhibition of acetylcholine esterase activity (Ryan and Byrne 1988 and Houghton *et al.* 2006) that leads to overstimulation of nervous system which result in neuro-toxicity and cellular death. Elevation in LDH is usually found in tissue breakdown and in cellular death, thus, LDH can be used as an indicator for cellular damage and cyto-toxicity of toxic agents (Jaiswal *et al.* 2013 and Ferri 2014). Furthermore, the basil oil had a strong effect on some biological aspects of *S. littoralis*, that significantly elongation both larval and pupal duration, decrease the pupal weight and pupation percentages as compared to control. That is could be due to the toxic components of the essential oil. Generally, the essential oils are also known to reduce growth of insects and act as antifeedants and moulting inhibitors (Arnason *et al.* 1989).

## CONCLUSION

The essential oil of basil leaves, *O. basilicum* was very rich in active components (30 chemical components)

and has a pronounced larvicidal potential against *S. littoralis* under laboratory bioassays. Further studies including the mode of action of the essential oil and the synergism with the biocides under the field condition are needed.

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## التركيب و الفعل الابادي للزيت العطري للريحان على يرقات دودة ورق القطن

هالة محمد إبراهيم ميعاد

معهد بحوث وقاية النباتات- مركز البحوث الزراعية - الدقى - الجيزة - مصر

استهدفت هذه الدراسة تقييم الكفاءة الحيوية للزيت العطري الطيار لأوراق نبات الريحان على يرقات دودة ورق القطن حديثة الفقس و أظهر التحليل الكيميائي لزيوت الريحان باستخدام جهاز جي سي ماس ان مركب لينالول (48.26%) و مركب يوكالبيبتول (9.21%) و كذلك استرجاع (5.16%) هي المكونات الرئيسية لهذا الزيت. وأوضحت النتائج ان لزيوت الريحان تأثيرا اباديا على يرقات دودة ورق القطن باستخدام التركيز القاتل من زيت الريحان لنصف عدد اليرقات، وكذلك تمت دراسة التأثيرات المتأخرة في الأطوار المتعاقبة و التي تمثلت في اطلالة معنوية لكلا العمرين اليرقي و العنزي مقارنة بالكنترول، كما تاثر النشاط الكيميائي الحيوي للحشرة نتيجة لاستخدام الزيت العطري فحدث انخفاض معنوي في نشاط انزيم أستيل كولين استريز و زيادة معنوية في انزيم لاكتات ديهيدروجينيز مقارنة بالكنترول.